

What we claim is:

1. A method of folding a denatured polypeptide, comprising the steps of:

(a) providing a polypeptide in an unfolded state which is capable of binding to a chaperonin;

5 (b) binding said polypeptide to said chaperonin to form a chaperonin-polypeptide complex for the folding of said polypeptide to its biologically active state; and

(c) exposing said chaperonin-polypeptide complex to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide.

10 2. The method of folding a denatured polypeptide of Claim 1 wherein said chaperonin is of the *Escherichia coli* GroE chaperonin family.

3. The method of folding polypeptides of Claim 2 in which the chaperonin is *E. coli* GroEL.

4. The method of folding polypeptides of Claim 1 in which the osmolyte is sucrose.

15 5. The method of folding polypeptides of Claim 1 in which the osmolyte is glycerol.

6. The method of folding polypeptides of Claim 1 in which the osmolyte is trimethylamine N-oxide.

7. The method of folding polypeptides of Claim 1 in which the osmolyte is potassium glutimate.

20 8. The method of folding polypeptides of Claim 1 in which the osmolyte is arginine.

9. The method of folding polypeptides of Claim 1 in which the osmolyte is betaine.

10. The method of folding polypeptides of Claim 1 in which the osmolyte is urea.

11. The method of folding polypeptides of Claim 1 in which the osmolyte is sarcosine.

12. The method of folding polypeptides of Claim 1 further comprising the step of promoting the folding of said polypeptide by the addition of a co-chaperonin to the chaperonin-polypeptide complex.

13. The method of folding polypeptides of Claim 1 further comprising a step of removing a metastable polypeptide folding intermediate prior to complete folding of the polypeptide and further stabilizing said metastable polypeptide.

14. The method of folding polypeptides of Claim 1 wherein said chaperonin is immobilized on an inert support.

15. The method of folding polypeptides of Claim 1 wherein the concentration of said osmolyte is sufficient to substantially prevent the aggregation of the unfolded polypeptides into unusable forms.

16. The method of folding polypeptides of Claim 1 wherein said unfolded polypeptide is incapable of being folded to its biologically active form by either a chaperonin or an osmolyte alone.

17. The method of folding a denatured polypeptide of Claim 1 wherein said method is conducted under controlled oxidation/reduction conditions.

18. The method of folding a denatured polypeptide of Claim 17 in which the oxidation/reduction conditions comprise an at least substantially anaerobic environment.

19. The method of folding a polypeptide of Claim 17 wherein said oxidation/reduction conditions are controlled by one or more redox agents selected from the group comprising glutathione, sulfhydryl and protein reduction systems.

20. The method of screening for an optimal folding environment for a denatured polypeptide, comprising the steps of:

(a) providing a polypeptide in an unfolded state which is capable of binding to a chaperonin;

(b) binding said polypeptide to said chaperonin to form chaperonin-polypeptide complexes for the folding of said polypeptide to its active state;

(c) providing a folding array having a plurality of elements with each element having comprising a different osmolyte solution therein;

(d) introducing a portion of said complexes to each of said elements in said array thereby exposing said complex to each of the osmolytes thereby promoting, to varying degrees, the folding of said polypeptide from its unfolded state to its folded state to yield a folded, biologically active polypeptide; and

(e) identifying the most efficient folding conditions for said polypeptide by measuring the yield of folded polypeptides within each element of said array.

21. The method of screening of Claim 20 wherein said chaperonin is of the *Escherichia coli* GroE chaperonin family.

22. The method of screening of Claim 21 in which the chaperonin is *E. coli* GroEL.

23. The method of screening of Claim 20 in which the osmolyte is sucrose.

24. The method of screening of Claim 20 in which the osmolyte is glycerol.

25. The method of screening of Claim 20 in which the osmolyte is trimethylamine N-oxide.

26. The method of screening of Claim 20 in which the osmolyte is potassium
5 glutamate.

27. The method of screening of Claim 20 in which the osmolyte is arginine.

28. The method of screening of Claim 20 in which the osmolyte is betaine.

29. The method of screening of Claim 20 in which the osmolyte is urea.

30. The method of screening of Claim 20 in which the osmolyte is sarcosine.

10 31. The method of screening of Claim 20 in which the osmolyte is L-proline.

32. The method of screening of Claim 20 further comprising the step of promoting
the folding of said polypeptide by the addition of a co-chaperonin to the chaperonin-polypeptide
complex.

15 33. The method of folding polypeptides of Claim 20 further comprising a step of
removing a metastable polypeptide folding intermediate prior to complete folding of the
polypeptide and further stabilizing said metastable polypeptide.

34. The method of folding polypeptides of Claim 20 wherein said chaperonin is
immobilized on an inert support.

20 35. The method of folding polypeptides of Claim 20 wherein the concentration of said
osmolyte is sufficient to substantially prevent the aggregation of the unfolded polypeptides into
unusable forms.

36. The method of folding polypeptides of Claim 20 wherein said unfolded polypeptide is incapable of being folded to its biologically active form by either a chaperonin or an osmolyte alone.

37. The method of folding a denatured polypeptide of Claim 20 wherein said method
5 is conducted under controlled oxidation/reduction conditions.

38. The method of folding a denatured polypeptide of Claim 37 in which the oxidation/reduction conditions comprise an at least substantially anaerobic environment.

39. The method of folding a polypeptide of Claim 37 wherein said
10 oxidation/reduction conditions are controlled by one or more redox agents selected from the group comprising glutathione, sulfhydryl and protein reduction systems.